Blood Cells, Molecules and Diseases 71 (2018) 75–76

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Letter to the Editor

Concomitant *CALR* and *LNK* mutations leading to essential thrombocythemia with erythrocytosis

ARTICLE INFO

Editor: Mohandas Narla

Keywords:
Myeloproliferative neoplasms
CALR
LNK
Essential thrombocythemia
Erythrocytosis

To the editor,

Myeloproliferative neoplasms (MPN) are characterized by a clonal expansion of mature blood cells and mainly include 3 diseases: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Their diagnosis has been simplified by the discovery of driver mutations (*JAK2*V617F, *MPL* and *CALR*) that allow detection of a molecular marker in more than 80% of cases. First described in 2005, the *JAK2*V617F mutation is found in more than 96% of PV and around half of ET and PMF. Mutations of *JAK2* exon 12 and *MPL* gene may also, rarely, be found in PV and TE or MPF, respectively. In 2013, a novel type of mutations affecting the *CALR* gene was described in *JAK2*V617F no mutated ET or PMF. *CALR* gene encodes calreticulin, a calcium-binding chaperone protein that promotes folding and quality control in the endoplasmic reticulum. All *CALR* mutations are insertion or deletion localized in the exon 9 that result in a frameshift with a common C-terminal sequence. First described only in ET and PMF, *CALR* frameshift mutations were then found in very few patients with erythrocytosis [1,2]. The mutated protein interacts with *MPL* receptor for JAK2/STAT5 pathway activation but not with the erythropoietin receptor [3]. When introduced in murine hematopoietic cells, it induces thrombocytosis without erythrocytosis. In a previous report, we identified 2 *CALR* mutated patients within a large cohort of unexplained polycythemia without *JAK2* mutation and reclassified them as (i) ET associated to a minimal polycythemia probably induced by chronic hypoxia and as (ii) an idiopathic erythrocytosis without any clue for MPN [4]. To date, screening for *CALR* mutation for the diagnosis of PV is not advisable and, if such mutation is found, it should not be considered as a convincing explanation for erythrocytosis.

Here we report a case of an 80-years old man that was referred to our lab for a chronic thrombocytosis. The patient had a history of heart arrhythmia and was treated by vitamin K antagonists. The initial examination showed no splenomegaly, mild leukocytosis (11.9×10^9/L), thrombocytosis (902×10^9/L) and normal hemoglobin and hematocrit (15.2 g/dL and 44.2% respectively). Nevertheless, a low serum erythropoietin (EPO) level (3.5 UI/L) motivated the measure of red cell mass that was increased (126%), without evidence for secondary erythrocytosis. This argues for the usefulness of the red mass measure in some cases of MPN as previously demonstrated. At the same time a classical molecular screening found no V617F or exon 12 mutation of *JAK2* but a previously described type 2-like mutation of *CALR* (c.1154_1155insATGTC; p.Glu386Cysfs*46; type 33 according Klampfl et al.). To better understand the molecular landscape of this case we performed a 64-genes custom next generation sequencing (Haloplex HS, Illumina NextSeq500) that confirmed the *CALR* mutation with a variant allele frequency (VAF) of 52%. It was associated to a *LNK* mutation (c.639C > A; p.Ser213Arg) with a VAF of 48% that was validated by Sanger sequencing. This mutation of LNK occurred in the “hotspot” of the exon 2 coding for the PH domain of the protein and was predicted to be deleterious by both SIFT, Polyphen2 and MutationTaster tools. The Ser213Arg mutation of LNK was previously described in a case of JAK2-negative erythrocytosis without somatic validation (COSM1685386) [5]. In this previous report the patient had a hemoglobin of 17 g/dL, a hematocrit of 50.4% and a low EPO level (4 UI/L) as in the patient reported herein. This *LNK* variant had a minor allele frequency of 0.063% in gnomAD database (rs111360561). For this reason we controlled the mutation on germinal DNA derived from nails and found that it was indeed a constitutional variant. LNK (SH2B3) is an adaptor protein that, after binding, inhibits JAK2 signaling. LNK deficient mice display marked changes of the hematopoiesis, including splenomegaly and a great increase of number of hematopoietic progenitors that have in vitro high proliferative capacities in part due to hypersensitivity to several cytokines [6]. Most of *LNK* mutations identified are missense mutations and occur predominantly in exon 2 coding for the PH domain of *LNK*. *LNK* mutations were found in MPN, especially at leukemic transformation and in idiopathic erythrocytosis (IE) [7]. In particular the mutation Glu208Gln, which is located near the mutation described herein, can be found in both MPN and IE and had a minor allele frequency of 0.099% in gnomAD database.

We therefore retained the diagnosis of *CALR*-mutated ET associated to a *LNK*-mutated constitutional mild erythrocytosis. Bone marrow biopsy was not feasible because of oral anticoagulation. The exclusion of PMF was based on absence of tear drop cells on the blood smear and

https://doi.org/10.1016/j.bcmd.2018.04.002
Received 19 April 2018; Accepted 21 April 2018
Available online 23 April 2018

a normal circulating CD34 cells count at 2/μL [8]. A treatment by hydroxycarbamide was started with a complete clinicohematological response at 18 months of follow-up.

This report emphasizes the interest of a NGS research for other mutations (such *LNK* or non-V617F *JAK2* mutations) when *CALR* MPN is associated to erythrocytosis. Other causes of erythrocytosis as high oxygen-affinity hemoglobin, *R-EPO* mutations or pathologic EPO production by tumors should also be considered in such cases.

Acknowledgements

This study is part of the clinical and biological database “FIMBANK” project supported by a grant of the French National Cancer Institute (INCa-BCB-2013).

Ethics approval and consent to participate

The patient was enrolled into the collection “Hémopathies Malignes” of the hospital of Angers who was approved by the CPP (Comité de Protection des Personnes) of Angers (France) Ouest II. A written informed consent was obtained from the patient for the use of clinical and biological data including DNA sequencing.

Competing interests

The authors declare that they have no competing interests.

References

[1] J. Broséus, J.-H. Park, S. Carillo, et al., Presence of calreticulin mutations in JAK2-negative polycythemia vera, Blood 124 (2014) 3964–3966 (blood-2014-06-583161), https://doi.org/10.1182/blood-2014-06-583161.

[2] S.E. Langabeer, K. Haslam, C.M. Flynn, Isolated erythrocytosis associated with a CALR mutation, Blood Cells Mol. Dis. (2017), http://dx.doi.org/10.1016/j.bcmd.2017.07.001.

[3] I. Chachoua, C. Pecquet, M. El-Khoury, et al., Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants, Blood (2015), http://dx.doi.org/10.1182/blood-2015-11-681932.

[4] A. Chauveau, O. Nibourel, S. Tondeur, et al., Absence of CALR mutations in JAK2-negative polycythemia, Haematologica 102 (2017) e15–e16, http://dx.doi.org/10.3324/haematol.2016.154799.

[5] A. Spolverini, L. Pieri, P. Guglielmelli, et al., Infrequent occurrence of mutations in the PH domain of LNK in patients with JAK2 mutation-negative “idiopathic” erythrocytosis, Haematologica 98 (2013) e101–e102, http://dx.doi.org/10.3324/haematol.2013.090175.

[6] L. Velazquez, Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice, J. Exp. Med. 195 (2002) 1599–1611, http://dx.doi.org/10.1084/jem.20011883.

[7] N. Maslah, B. Cassinat, E. Verger, et al., The role of LNK/SH2B3 genetic alterations in myeloproliferative neoplasms and other hematological disorders, Leukemia 31 (2017) 1661–1670, http://dx.doi.org/10.1038/leu.2017.139.

[8] C. Orvain, D. Luque Paz, I. Dobo, et al., Circulating Cd34+ cell count differentiates primary myelofibrosis from other Philadelphia-negative myeloproliferative neoplasms: a pragmatic study, Ann. Hematol. 95 (2016) 1819–1823, http://dx.doi.org/10.1007/s00277-016-2784-x.

Damien Luque Paz[a,b,c,d], Françoise Boyer[e], Annaëlle Beucher[a], Anne Bouvier[a,c,d], Rebecca Jouanneau-Courville[a,d], Philippe Guardiola[b,c,d,f], Diane Lambert[f], Yves Delneste[c], Mathilde Hunault[b,c,d,e], Odile Blanchet[a,b,c,d,g], Valérie Ugo[a,b,c,d,*]

[a] *CHU Angers, Laboratoire d'Hématologie, Institut de Biologie en Santé, Angers, France*

[b] *Université d'Angers, UFR Santé, Angers, France*

[c] *CRCINA, INSERM, Université de Nantes, Université d'Angers, Angers, France*

[d] *Fédération Hospitalo-Universitaire (FHU) GOAL, ‘Grand Ouest Against Leukemia’, France*

[e] *CHU Angers, Service des Maladies du Sang, Angers, France*

[f] *CHU Angers, Service de Génomique Onco-Hématologique, Angers, France*

[g] *CHU Angers, Centre de Ressources Biologiques, BB-0033-00038, Angers, France*

E-mail address: valerie.ugo@univ-angers.fr

[*] Corresponding author at: Laboratoire d'Hématologie, Institut de Biologie en Santé, CHU Angers, 4 rue Larrey, 49933 Angers, France.